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SYNTHETIC PEPTIDE VACCINES FOR THE CONTROL OF ARENAVIRUS INFECTONS

ANNUAL REPORT

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Arenaviruses are endemic on both the African and South American continents and represent significant public health hazards. Prophylactic immunization, precise diagnostic methods, and effective treatment protocols are not currently available. We are using genetic cloning methods to develop an effective vaccine against arenaviruses. Developmental studies have been carried out and techniques established with the prototype arenavirus, lymphocytic choriomeningitis virus (LCMV), and these methods can now be applied for rapid development and evaluation of vaccines against the pathogenic arenaviruses Lassa, Junin, and Machupo. Using techniques of peptide and immunochemistry we have identified and mapped the gene products of the L and S-RNA (OVER)

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Abstract

Arenaviruses are endemic on both the African and South American continents and represent significant public health hazards. Prophylactic immunization, precise diagnostic methods, and effective treatment protocols are not currently available. We are using genetic cloning methods to develop an effective vaccine against arenaviruses. Developmental studies have been carried out and techniques established with the prototype arenavirus, lymphocytic choriomeningitis virus (LCMV), and these methods can now be applied for rapid development and evaluation of vaccines against the pathogenic arenaviruses Lassa, Junin, and Machupo. Using techniques of peptide and immunochemistry we have identified and mapped the gene products of the L and S-RNA segments of LCMV and mapped the important immunogenic regions of the viral glycoproteins. The LCMV genomic RNAs have been cloned and primary sequences of the RNAs and their gene products are being completed. Synthetic peptides corresponding to immunogenic regions of the viral structural proteins are being synthesized and will be evaluated for the ability to induce immune responses in experimental animals. Experimental approaches to immunization based upon synthetic peptides and polypeptides, vaccinia virus vectors containing LCMV genes, and anti-idiotypic antibodies will be explored. Experimental approaches to immunotherapy for acute arenavirus infections will also be investigated using cloned cytotoxic T-lymphocytes and neutralizing monoclonal antibodies in attempts to modify the course of acute disease. Finally, monoclonal antibodies and cDNA probes against defined type specific and common determinants and sequences will be made in order to facilitate precise diagnosis of arenaviral diseases.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

The investigators have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

Table of Contents

A. Introduction and Overview

During the contract year 1 Aug 1988 - 31 Jul 1989 we have focused our effort on the following issues related to contract C6234:

1. Mechanism and specificity of antibody mediated protection from lethal acute disease induced by lymphocytic choriomeningitis virus. We established a model of passive protection of mice from acute challenge infection based solely upon transfer of monoclonal antibodies to defined epitopes on the GP-1 glycoprotein. Requirements for timing of the antibody transfer were explored in terms of viral replication in tissues, survival, and effect upon the cytotoxic T-cell response to virus.

2. In order to understand early stages in virus-cell interaction we established a virus binding assay utilizing ^{35}S methionine labeled, purified LCMV and binding the virus to susceptible Vero cells. Kinetics and temperature dependence of binding were studied as well as the requirements for metabolic energy. We utilized our panel of monoclonal antibodies to inhibit virus binding to attempt to define epitopic regions of GP-1 which were spatially related to receptor binding regions of that protein.

3. In order to more precisely define the epitopes critical for protection and the topographical structures recognized by antibodies which inhibited virus binding, we prepared a series of synthetic peptides representing overlapping sequences from GP-C residues 59-498 and assessed monoclonal antibody binding to these peptides.

4. Studies to delineate the macromolecular structure of the viral glycoprotein spike by nearest neighbor analysis were begun during this project year and will be carried forth into the next year. Initial experiments were based on protein cross-linking using bifunctional reagents such as dimethyl subrimate (DMS), performed in order to define the oligomeric structure of the glycoprotein spike, a fundamental property of the viral architecture.

5. During this interval we also made two trips (Dec 1988 and Apr 1989) to Ft. Detrick for the purpose of extracting Lassa RNA for molecular cloning. These trips were abortive. In the first, a centrifuge failed in the P-4 suite in the final stages of RNA purification, resulting in loss of the material. In the second case, despite a successful and well-controlled extraction, no Lassa RNA was obtained, indicating that insufficient virus was present in the "purified virus preparation" prepared at USAMRIID prior to our arrival. These problems were detailed in quarterly reports of 15 Feb 89 and 15 May 89.

B. Mechanism and Specificity of Antibody Mediated Protection from Acute LCMV Disease

It is highly desirable in the design of a vaccine to have prior knowledge of the protective mechanisms operative in acquired resistance. In the case of arenavirus disease, anecdotal evidence suggested that the protective role of humoral antibody had been largely underestimated. Therefore we investigated the mechanism of monoclonal antibody (MAb) mediated protection agaist LCMV-induced acute CNS disease. The basic protocol was to passively administer the MAb under study intraperitoneally from 1 day before to 2 days after intracerebral (ic) challenge with 500 pfu (ca. >1,000 LD_{50}) of LCMV-Arm. Virgin mice challenged in this way always died by the sixth to seventh day post-infection. Table 1 illustrates the experimental data for in vivo protective effect of antibody in acute LCM disease. Note from the data that 3 MAbs, 2-11.10, 258.2 and 67.2, protected mice against lethal virus challenge. All were GP-1 specific, but significantly, only two of these MAb neutralize virus in vitro. MAb 2-11.10 was effective when administered as late as two days (20% mortality) after infection, indicating an inhibition of spread of virus infection. Table 2 illustrates the observation that protected mice show 2 log or greater reductions in virus burden in their brains, and do not become virus carriers, as indicated by clearance at days 14 and 30. Table 3 illustrates that relative to their non-antibody treated cohorts, the antibody protected mice showed lower levels of CTL activity, which may account for lower inflammatory responses and survival of these mice.

These findings are important in that they establish that a pre-existing humoral antibody to LCM virus is protective, and that epitopes on GP-1 are crucial. Protection did not require in vitro virus neutralizing activity however. The latter finding may be particularly important since the presence of neutralizing antibody to Lassa virus in convalescent patients and animals is difficult to demonstrate. These data have been submitted to Nature as a brief report (Wright and Buchmeier, Nature, submitted).

C. Virus Binding Assay

It is of interest to identify the protective epitopes recognized by MAb to GP-1. Therefore we initiated studies of the mechanism of virus neutralization and attempted to identify these epitopes at the molecular level. Since we have already mapped the 2-11.10 epitope to amino acid 173 of GP-1, we focused on 1) the biological activity of these MAbs in binding inhibition, and 2) mapping the sequential (peptide) epitope 67.2 (section D below).

A binding assay was established to study the in vitro inhibitory effect of antibody on LCMV replication in vitro and in vivo. Binding of radiolabeled (^{35}S) LCMV showed saturation kinetics at both 4^{o} and 37^{o} with approximately 3-fold more virus bound at 37^{o}, suggesting that internalization follows rapidly at 37^{o} (Fig.

Table 1. Protection against intracranial challenge with LCMV by transfer of monoclonal antibodies.

MAb	Specificity	ELISA	Neut. Titre	Day of Transfer	%Mortality (n)
211.10	GP-1	204,000	+	-1,0	4 (24)
258.2	GP-1	12,800	+	-1,0	24 (15)
67.2	GP-1	3,200	-	-1,0	0 (11)
9-7.9	GP-2	800	-	-1,0	90 (10)
1-1.3/ 10-7.5	NP	204,000	-	-1,0	94 (16)
MHV MAb	-		-	-1,0	90 (18)
211.10	GP-1		+	0	0 (5)
				+1	0 (10)
				+2	20 (5)
				+3	80 (5)
Saline			-	0	88 (9)

Female 4-6 week old Balb/c ByJ mice were given 0.2 ml ascites intraperitoneally (i.p.) on the specified days, where day 0 is the day of challenge with 500 PFU LCMV, strain Armstrong intracranially (i.e.). All mice were observed for at least 30 days. 500 PFU is equal to 263 LD50 of the Arm stock used in these experiments.

Table 2. Virus clearance from brains of mice challenged with LCMV i.c.

MAb	log10 PFU per g brain				
	day 1	day 4	day 6/7	day 14	day 30
Expt 1					
2.11.10	<2.0	<2.0	n.d.	<2.0	<2.0
	<2.0	<2.0		<2.0	<2.0
	<2.0	4.3			<2.0
	<2.0	4.3			<2.0
	<2.0	3.2			
10-7.5	<2.0	7.4	n.d.		
	4.0	5.8			
	3.5	6.0			
	4.5	5.7			
	4.3	6.3			
Expt 2					
2.11.10	<2.0	4.4	<2.0		
	<2.0	4.7	<2.0		
	<2.0	4.8	<2.0		
	<2.0	4.9	<2.0		
	<2.0	5.1	4.5		
9-7.9	<2.0	6.9	<2.0		
	<2.0	6.9	<2.0		
	<2.0	6.4	6.6		
	<2.0	5.8	6.4		
	<2.0	5.4	3.7		

Groups of 4-6-week old female Balb/c mice were given 0.2 ml ascites intraperitoneally, then challenged with 1000 pfu (Expt 1) or 500 pfu (Expt 2) i.c. 24 hrs later. Brains were collected at specified times, frozen and assayed for infectious virus on Vero cell monolayers. Unprotected mice were usually dead by day 7.

Table 3. Cytotoxic T-cell responses to LCMV in protected mice.

Effector		Percent ^{51}Chromium Release $H\text{-}2^d$-LCMV	$H\text{-}2^d$ Mock	$H\text{-}2^b$-LCMV	
Day 7-i.p.		68.5	0	4.0	Not protected
Day 7-i.c.	83.6	52.8	1.3	0.9	
Day 7-i.c.	67.2	16.0	1.2	0	
Day 9-i.c.	67.2	16.9	0	0.4	Protected
Day 11-i.c.	67.2	12.8	1.8	0	
Day 14-i.c.	67.2	3.1	0.9	0	
H-2b Control		-	-	66.2	

Cytotoxic activity was measured in a ^{51}Chromium release assay in splenocytes, collected from mice given MAb and infected i.c. ^{51}Chromium labelled targets were: $H\text{-}2^d$ - Balb/c Cl7, $H\text{-}2^b$ - MC57/MEF.

1). In the presence of 0.1% sodium azide, an inhibitor of cellular energy production, an intermediate level between the 4° and 37° saturation curves was observed. The full library of MAbs against GP-1 of LCMV was tested for ability to inhibit this virus binding (Table 4), and it was noted that the most efficient inhibition of binding was achieved with MAbs 36.1, 67.2 and 67.5 (sister clones) and 2-11.10. The latter two MAbs were shown previously to be protective if administered passively to mice either prior to or after LCMV infection. Thus epitopes have been defined which elicit antibody which inhibits virus-cell interaction. These correspond in some cases (e.g. 2-11.10 and WE 67.2) to protective epitopes and as such are logical targets for vaccination.

D. Mapping of Peptide Epitopes on GP-C

In a continuation of our efforts to map and characterize the topography of GP-C and its cleavage products GP-1 and GP-2, synthetic peptides derived from the complete sequence of the GP-C precursor were made and characterized (Fig. 2). We screened the GP-1 peptide sequences against our panel of MAb to GP-1 by ELISA, and the binding patterns of these antibodies against the peptides are illustrated in Table 5. Note that one peptide in particular, 201-216, gave very high background binding of several monoclonals as indicated in matrix scores of 5-9 or 10 (asterisk). Furthermore, some monoclonals such as 9-7.9 gave high binding scores against several peptides in spite of the fact that this antibody recognizes a GP-2 sequence (GP-C 370-382) (Weber and Buchmeier, Virology 164:30-38, 1988). On the basis of these observations and other inconsistencies in the results, it was decided that this approach was not sufficiently reliable to map epitopic sites.

E. Macromolecular Structure of the Glycoprotein Spike

Dr. John Burns, a molecular virologist experienced in protein chemistry, joined my laboratory group during this year to continue work on the structure of the arenavirus spike. Dr. Burns has performed experiments to look in more detail at the biosynthesis and folding of the LCMV glycoproteins as a model for Lassa. We feel that this comparison is justified in view of the overall high degree amino acid similarity (>77%) between the glycoproteins of these two viruses, and their similar biology (Fig. 3). Dr. Burns has performed two kinds of experiments to date. The first was precisely timed pulse chase studies to define the time required for synthesis and processing of GP-C to GP-1 and GP-2. By pulse labeling for 5 minutes and chasing for various intervals, it was shown that 75 minutes elapsed between GP-C biosynthesis and proteolytic cleavage. By inhibiting intracellular transport at low temperatures (16°C, 25°C), it was shown that cleavage occurred in the Golgi apparatus. These results, as well as the results of other transport studies, were reported in a manuscript submitted to Virology.

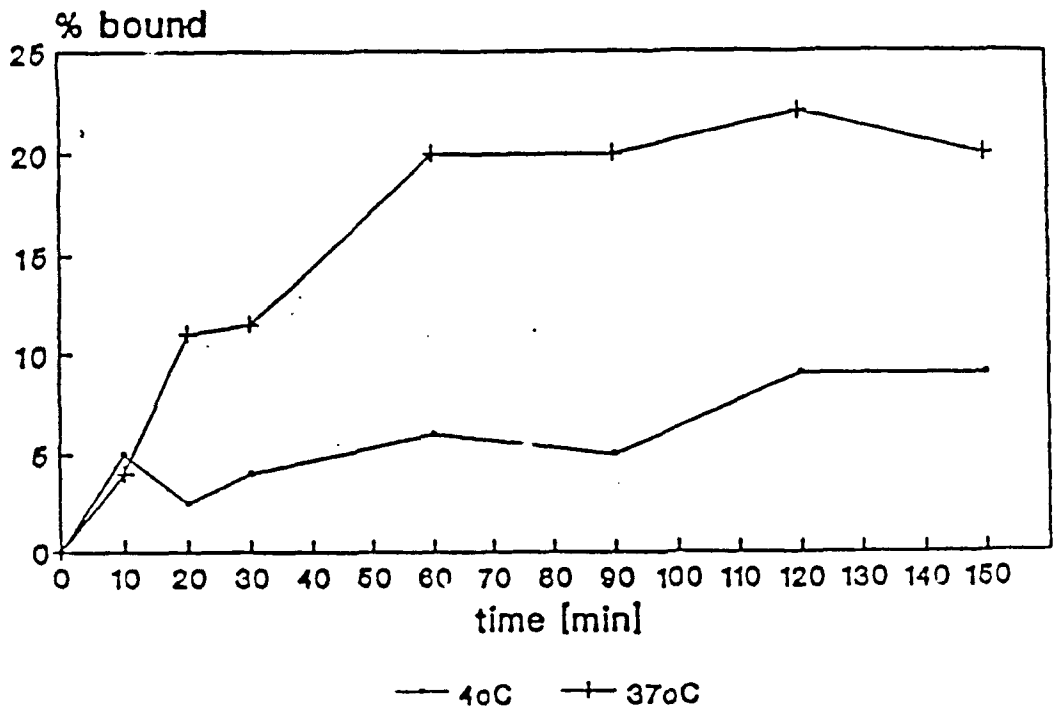


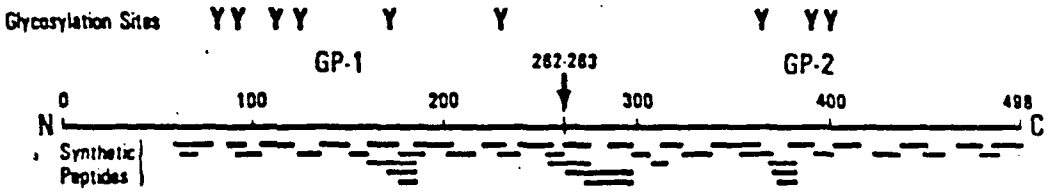
Fig. 1 : Binding curves of radiolabeled LCMV-Arm 4 to Vero cells.

Table 4. Effects of monoclonal antibody or polyclonal antisera on the binding of labeled LCMV-Arm 4 to Vero cells.

			Inhibition					
			37°C			4°C		
MAb	Epitope	Neutral.	1:50	1:100	1:1000	1:50	1:100	1:1000
WE-36.1	GP-1A	+	++	++	+	+++	++	+
WE-258.4	GP-1A	+	-	-	-	-	-	-
WE-197.1	GP-1A	+	-	-	-	-	-	-
WE-6.2	GP-1A	+	-	-	-	-	-	-
WE-40	GP-1A	+ (WE)	+	+	-	+	-	-
WE-327.3	GP-1B	-	-	-	-	-	-	-
WE-2.9	GP-1B	-	-	-	-	-	-	-
WE-18.8	GP-1C	-	-	-	-	-	-	-
WE-67.5	GP-1C	-	++	+	-	++	+	-
WE-67.2	GP-1C	-	++	+	-	++	+	+
2.11.10	GP-1D	+ (Arm)	++	+	-	++	+	+
WE-33.6	GP-2A	-	+	-	-	+	+	-
WE-83.6	GP-2A	-	+	-	-	-	-	-
Arm 9.7.9	GP-2B	-	+	-	-	+	-	-
WE 1-1.3	NP	-	-	-	-	-	-	-
MHV 5B11.5	-	-	-	-	-	-	-	-
Polyclonal								
Guinea pig	LCMV	+	+	+	-	+	-	+
Guinea pig	normal	-	-	-	-	-	-	-

Note: +++ inhibition between 100-75 %; ++ inhibition between 75-50%; + inhibition between 50-25% and - inhibition between 25-0%.

Fig. 2. **SYNTHETIC PEPTIDES FROM LCMV GP-C SEQUENCE**



LCMV - Peptides

	GP-C	Sequence
GP-1	59-79(83B)	MYGLKGPDIYKGVYQFKSVEF
	62-71	LKGPDIYKGV
	87-96	TMPNACSANN
	92-104	CSANNSHHYISMG
	104-121	(CGG)TSGLELTFTNDSIISHN
	123-135	CNLTSAFNKKTFD
	125-145(84B)	LTSAFNKKTFDHTLMSIVSSV
	135-150	(CGG)DHTLMSIVSSLHLSIR
	150-160	RGNSNYKAVSC
	160-176	CDFNNGITIQYNLTFSD
	160-185	CDFNNGITIQYNLAFSDEQSAQSQC
	170-184	YNLAFSDEQSAQSQC
	176-184	DEQSAWSQC
	176-189	DAQSAQSQCRTFRG
	184-205	CRTFRGRVLDMFRTAFGGKYMR
	204-216	GGKYMRSGWGWTGSDGK
	220-232	CSQTSYQYLIIQN
	228-239(H21B)	LIIQNGTWENHC
	239-256	CTYAGPFGMSRILLSQEK
PANS CLEAVAGE SITE	253-262	SQEKTKFFTR
	254-275	SQEKTKFTRRLAGTFTWTLSDS
	262-275	RRLAGTFTWTLSDS
GP-2	272-285(H22B)	LSDSSGVENPGGYC
	272-285	
	285-297	CLTKWMILAAELK
	297-306	CFGNTAVAK
	307-315	CNVNHDAEF
	312-232(47B)	DAEFCDMLRLID
	323-338	(CGG)DYNKAALSKFKEDVES
	338-363	(CGG)SALHLFKTTVNSLISD
	353-370	DQLLMRNHLRDLMGVPYC
	368-382	PYCNYSKFWYLEHAK
	370-382	CNYSKFWYLEHAK
	378-391(48B)	LEHAKTGETSVPKC
	391-401	CWLVTNGSYLN
	401-415	(CGG)NEIHFSDQIEQEADN
	422-435	(CGG)RKDYIKRQGSTPLA
	436-450	(CGG)ALMDLLMFSTSAYLIS
	448-458	LVSIFLHLVKIGGC
	465-478	(C)KGGSCPKPHRLTNK
	477-487	NKGICSCGAFK
	483-498	CGAFKVPGVKTIWKRR

Table 5. Results of Comparison of Monoclonal Antibodies versus Synthetic Peptides following ELISA

	ANTIBODY																		
GP-C	Blank	WE 36.1	WE 258.4	WE 197.1	WE 6.2	WE 40	WE 327.3	WE 2.9	WE 18.8	WE 67.5	WE 67.2	2.11.10	WE 33.6	WE 83.6	Arm 9.7.9	WE 2.9	WE 258.4 (5 ul)	WE 327.3 (5 ul)	WE 1.1.3	G.P. LCMV
59-79	0	2	2	0	0	3	3	0	0	0	0	0	0	0	1	0	0	1	0	*
62-71a	0	0	2	0	0	0	5	0	0	0	0	0	0	0	0	0	0	1	0	0
62-71b	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
87-96	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0
92-104	0	0	3	0	0	0	4	0	0	0	1	1	0	0	1	0	1	1	0	0
104-121	0	0	9	1	1	1	*	1	1	1	2	3	1	0	4	0	2	3	0	5
123-135	0	0	4	0	0	0	4	0	0	0	1	1	0	0	0	0	0	1	0	1
135-150	0	0	2	0	0	0	3	0	0	0	0	1	0	0	1	0	0	1	0	2
150-160	0	0	2	0	0	0	4	0	0	4	0	0	0	0	0	0	0	1	0	2
160-185	0	0	5	1	0	0	6	0	1	0	1	2	1	1	0	0	2	3	0	3
170-184	0	0	3	1	0	0	4	1	0	0	1	0	0	0	2	0	1	1	0	0
176-184	0	0	3	0	0	0	4	0	0	0	0	1	0	0	2	0	1	1	0	0
176-189	0	0	3	0	0	0	3	0	0	0	0	0	0	2	1	0	1	1	1	*
184-205	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	4
201-216	0	0	*	1	1	7	*	*	0	0	4	1	5	9	*	*	*	9	1	*
220-232	0	0	2	0	0	0	3	0	0	0	0	0	0	0	2	0	1	1	1	2
228-239	0	0	3	0	0	0	3	0	0	0	0	0	0	0	6	0	3	2	0	2
239-256	0	0	4	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	1
253-262	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0
254-275	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	3
262-275	0	0	1	0	0	0	1	0	0	0	0	0	0	0	3	0	2	1	0	3
272-285	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
370-382	-	-	-	-	-	-	-	-	-	-	0	0	9	9	9	-	0	0	0	-

Note: The original optical density scale from 0 to 2.0 is transferred in to matrix scale from 0 to * (10), thus a score of 1 corresponds to an OD of 0.2, etc.

Fig. 3. Amino Acid Similarity Between LCM-Arm and Lassa-Jos GP-C Glycoprotein precursors.

```
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(No documentation)

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Percent Similarity: 77.160  Percent Identity: 61.523

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        : : |  ::|:  ::.||| ||||||||||.| :.|||||||:||::.||
    240 GYLGLLSQRTRDIYISRRLLGTFTWTLSDSEGKDTPGGYCLTRWMLIEAE 289

    296 LKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDVESALHLFKT 345
        |||||||||||||| .||.|||||||:|:||.|: ::|.:.: .::|:..
    290 LKCFGNTAVAKCNEKHDEEFCDMLRLFDFNKQAIQRLKAEAQMSIQLINK 339

    346 TVNSLISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVT 395
        .||.||.|||:|:|||||:||:|||||||:|||:|..|| ||:|||||.
    340 AVNALINDQLIMKNHLRDIMGIPYCNYSKYWYLNHTTTGRTSLPKCWLVS 389

    396 NGSYLNETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTS 445
        ||||||||||||:|||:|||||||||.|:|:.|||.|||:|:|||::|||
    390 NGSYLNETHFSDDIEQQADNMITEMLQKEYMERQGKTPLGLVDLFVFSTS 439

    446 AYLVSIFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVW 495
         ||:|||||||||||||| | ||||||||.:.|||||| :| |||.. |
    440 FYLISIFLHLVKIPTHRHIVGKSCPKPHRLNHMGICSCGLYKQPGVPVKW 489

    496 KR 497
        ||
    490 KR 491
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In order to better define the GP-1 and GP-2 structural proteins more accurately, we utilized the algorithm of von Heijne to predict the most likely sites of signal peptide cleavage for the arenaviruses LCMV, Lassa, Pichinde and Tacaribe, for which sequence is available. Table 6 illustrates the most likely cleavage sites and their von Heijne scores. Note that in each case an extraordinarily long signal sequence of ca. 58 amino acids is predicted at the amino terminus of GP-1. We have utilized direct protein microsequencing to confirm this result and to identify the N-terminal residue of GP-2 for LCMV (Fig. 4). The sequence MYGLK at the N-terminus of GP-2 corresponds to residues 59-63 of the GP-C open reading frame, precisely where cleavage was predicted by the algorithm. The GP-2 sequence GTFTWT matched residues 266-271 of GP-C and was two residues to the carboxyl side of the predicted ARG-ARG cleavage recognition site. In the next year we will continue these studies as well as additional structural work on the spike.

The second series of experiments dealt with the molecular association between GP-1 and other virion proteins. Cross linking of virions with the bivalent 11 Angstrom cross linking reagent dimethyl subrimate DMS revealed that GP-1 exists as homo-oligomers of 1x, 2x, 3x and 4x composition, where x = GP-1 monomer. Thus GP-1 is in all likelihood a tetrameric structure. GP-2 in contrast formed homodimers (GP-2:GP-2) and heterodimers (GP-2:NP) but was not associated with GP-1.

F. Plans for the Coming Year

During the contract year 1 Aug 89 - 31 Jul 1990 we will 1) continue our work to define the mechanism of protection by antibody, 2) continue to refine our work on the structure of the arenavirus spike, and 3) define a scheme for purification of the spike for use as a subunit immunogen. In addition, we will use refined in situ hybridization techniques to study the distribution of LCMV in the persistently infected mouse at the cellular and tissue level. It is expected that these methods will be useful in studies of human and animal disease induced by arenavirus infection.

Table 6.

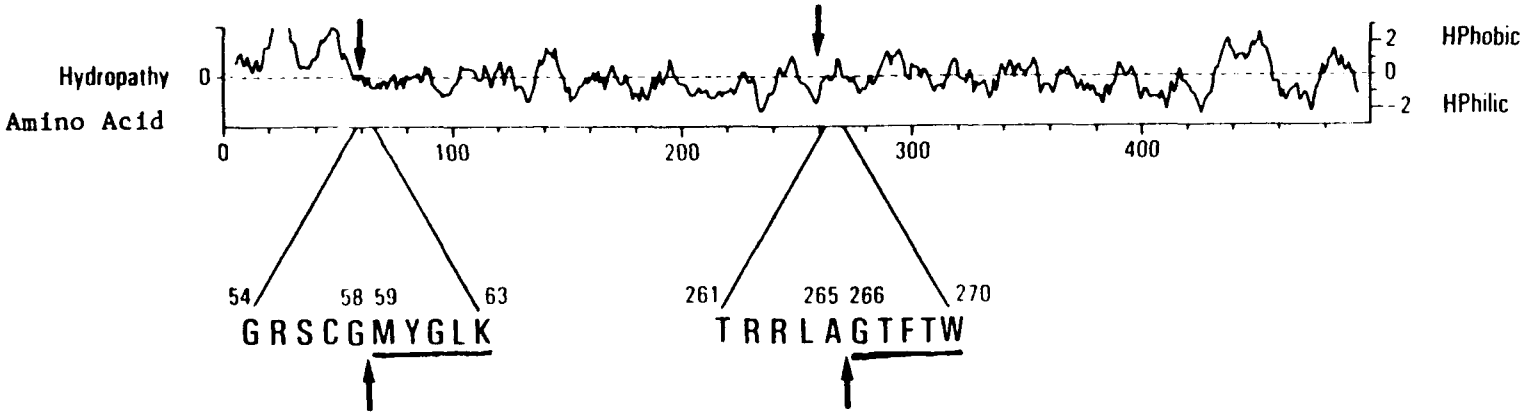
PREDICTED SIGNAL PEPTIDASE CLEAVAGE SITE* ON ARENAVIRUS GPC

	LCMV (Arm)		LCMV (WE)		Lassa		Tacaribe		Pichinde	
Rank	Residue	Score	Residue	Score	Residue	Score	Residue	Score	Residue	Score
1	58	8.305	58	7.320	58	6.332	58	8.106	16	5.576
2	56	5.954	56	7.017	56	5.433	56	4.758	56	3.489
3	34	4.816	54	4.594	34	5.006	53	4.561	59	3.367

*computer analysis of amino acid sequences based on algorithm of von Heijne (Fazakerley and Ross, 1988).

Fig. 4.

LCMV ARMSTRONG GPC CLEAVAGE SITES



Note: Amino terminal sequences MYGLK for GP-1 and GTFTWT were confirmed by microsequencing.

Appendix 1: Publications supported by this document, 1988-89

Published Papers

*Wright, K. E., M. S. Salvato, and M. J. Buchmeier. 1989. Neutralizing epitopes of lymphocytic choriomeningitis virus are conformational and require both glycosylation and disulfide bonds for expression. Virology 171:417-426.

*previously listed as papers in press.

Papers Submitted

Wright, K. E., R. C. Spiro, J. W. Burns and M. J. Buchmeier. Post-translational processing of the glycoproteins of lymphocytic choriomeningitis virus. Virology, 1989, submitted.

Wright, K. E. and M. J. Buchmeier. Antiviral antibodies attenuate T-cell mediated immunopathology following acute lymphocytic choriomeningitis virus infection. Nature, 1989, submitted.

Fuller-Pace, F. V. and P. J. Southern. Detection of viral-specific RNA-dependent RNA polymerase activity in extracts from cells infected with lymphocytic choriomeningitis virus: in vitro synthesis of full-length viral RNA species, J. Virol., submitted.